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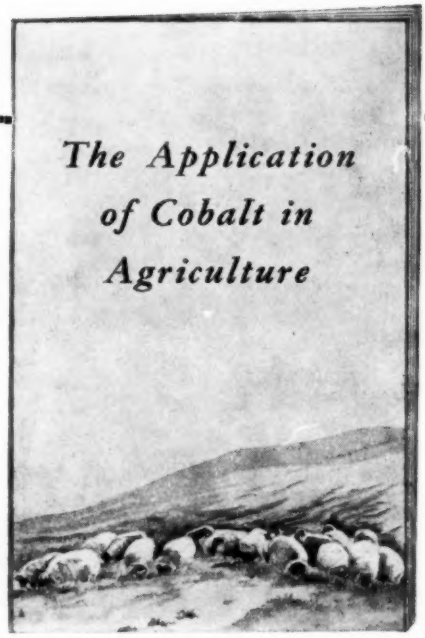


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## Pig Insemination Technique

BY

**J. L. HANCOCK**

**A R.C. Animal Breeding Research Organisation, Edinburgh**

*SUMMARY.—The frequency of fertilisation shown by ova recovered from sows after insemination has been used to measure the efficiency of 2 insemination techniques.*

*With the first method (A) a flexible polythene catheter, guarded within a stout polythene sound, is passed up to the level of the external os and is then passed beyond the sound through the cervical canal into the uterus. The site reached for insemination was recorded and the fertility of inseminations made at the 3 sites was examined.*

*Of 51 sows inseminated, 40 (74.5 per cent.) had fertilised ova; 64.6 per cent. of all ova recovered were fertilised (84.1 per cent. fertilised ova from "fertile" sows). Insemination into the uterus was done in 27 sows, 26 of these (96.3 per cent.) had fertilised ova; 84.1 per cent. of all ova from sows inseminated into the uterus were fertilised (87.7 per cent. ova from "fertile" sows).*

*Five of 10 sows inseminated intracervically conceived (52.3 per cent. of all ova were fertilised; all ova from "fertile" sows, fertilised); 8 of 14 sows inseminated into the cervical os ("vaginally") conceived (33.3 per cent. of all ova fertilised; 59.8 per cent. fertilised ova from "fertile" sows).*

*Of a total of 12 sows inseminated by the method of Aamdal and Hogset (method B) 10 conceived (59.2 per cent. of all ova fertilised; 71.4 per cent. fertilised ova from "fertile" sows).*

*In one experiment where alternate sows were inseminated by method A and method B, all of 9 sows inseminated by method A conceived (95.4 per cent. ova fertilised). Seven of the 9 sows inseminated by method B conceived (60.1 per cent. of all ova fertilised; 73.1 per cent. fertilised ova from "fertile" sows).*

*The effects of sow differences and of variation in insemination procedure on the site reached during insemination, are examined. Most of the variation in fertility between inseminations was due to variations in the site reached for insemination.*

IN an earlier investigation (Hancock, 1958a) it was found that the frequency of fertilisation among ova recovered from sows inseminated with fresh undiluted semen was lower (47 per cent.) than among ova from naturally mated sows (91 per cent.). It was concluded that the technique used to deposit the semen into the sow was inefficient.

It seemed likely that the poor results reported earlier were due to failure to place the semen into the uterus. The need to do this is now widely accepted and some of the equipment currently in use in commercial practice has been designed with this end in view (Aamdal & Hogset, 1957; Niwa, 1958). The investigations reported now were undertaken to examine more closely the efficiency of pig insemination technique and to seek improved methods.

### Material and Methods

Inbred and outbred Large White sows and their crosses and a few outbred Wessex sows all from the Organisation's pig breeding unit were used. Large White boars from the same source were used. The sows were allotted for use in these investigations as they became surplus but were otherwise unselected. All sows had farrowed 50 to 60 days previously. No gilts were available for insemination. After weaning sows were tried once daily with a vasectomised boar for evidence of heat. The day of first acceptance was regarded as the day of onset of heat. In this paper this day is called "day O," the day after onset of heat is called "day 1." In most cases service was prevented. This was sometimes judged to be neces-

sary because several sows were near heat on the same day and it was important that the boar should try them all.

All but one of the 6 fertile boars were mated naturally to sows; the results of the matings were measured in the same way as the results of artificial inseminations. For inseminations semen was collected and used within 75 minutes of collection after removal of the gel fraction by filtration through glass wool. The semen was stored at 30° C. to 37° C. up to the time of insemination. All semen collections were made by Mr. R. G. Griffiths. The semen was examined for motility before use and morphological examinations were made subsequently. No semen was rejected as unfit for insemination.

There was considerable variation in the procedure followed for the trial and insemination of sows. Initially, most artificial inseminations were made on day O but in view of the possibility that failures to penetrate the cervix might be due to some effect of the previous sterile mating, later matings were made on day 1.

Sows were inseminated while restrained in a crate or without restraint in an open paddock. Originally, restraint was usually applied for the sake of convenience rather than from necessity, but later in the experiments sows were inseminated under restraint only if necessary.

*Insemination Equipment*

The results of inseminations with two types of equipment (A and B) are dealt with here. The equipment is described below.

A. This instrument is basically the same as that used by Holt (1957) and by Manton (1957); it is illustrated in Fig. 1. It consists of a tubular polythene sound (length 35 cm.; diameter 14 mm.) closed at one end by a plug of polythene which is drilled to admit a soft flexible polythene pipette (length 52 cm.; outside diameter 3 mm.—Portland Plastics size 54A). When in use the pipette is stiffened with a 65 cm. length of copper-coated steel wire (welding rod, diameter 2 mm.).

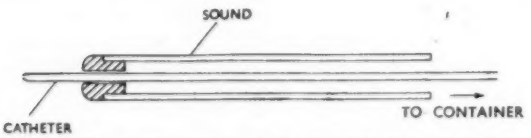


FIG. 1.—The equipment used for insemination of pigs by Method A.

B. This is the cuffed pipette described by Aamdal and Hogset (1957).

For delivery of the semen both pipettes were attached by a rubber connexion to a collapsible polythene container and the semen was delivered by squeezing the container. For most inseminations the containers supplied with the Norwegian pattern pipette (method B) were used but a few inseminations were made with containers made in the laboratory from polythene tubing (external diameter 8 mm; bore 3 mm.). These were made by sealing the end of the tube with hot forceps and blowing a bulb of about 100 c.c. capacity at the end after softening by immersion in hot glycerol (I am indebted to Dr. A. D. Bangham for the method).

*Insemination Technique*

For insemination (with both methods) the pipette was freely lubricated with liquid paraffin B.P. delivered from a polythene wash-bottle. The pipette (guarded within its sound in method A) was thrust forward and upward to avoid the urethral orifice and its tip was placed as near as could be judged into the cervical os. This seems to be the most critical point in insemination, whether with method A or with method B; with experience it is possible to distinguish between the resistance offered by the cervical os and that offered by a fold of the vaginal wall. There is also (with method A) a characteristic "feel" about the cervical os when it meets the inner pipette; it gives the impression of being sticky—the sensation is quite different from that experienced when the pipette meets the cow's cervix during insemination. With method B the semen was injected with the tip of the pipette within the cervical os, the cuff being inflated before injection.

With method A the tip of the sound was held firmly in contact with the cervical os while attempts were made to pass the inner pipette through the cervical canal. To do this, the wire was withdrawn from the pipette for a few millimetres to give it a flexible tip and the pipette with its wire was then advanced, being simultaneously rotated on its long axis. When successful the pipette tip was felt to move in a series of steps over the cervical folds until it could be moved freely apparently within the cavity of the uterus. The degree of success at penetration was judged on these grounds and from the length of pipette found protruding beyond the end of the sound after its withdrawal. "Uterine" insemination was recorded as successfully achieved when the length of the freely protruding portion of the pipette was judged to be not less than 12 cm. For "cervical" insemination the length was 2 to 12 cm. and for "vaginal" insemination it was less than 2 cm. So-called "vaginal" inseminations were in almost all cases probably made into the cervical os.

To deliver the semen the wire was withdrawn from the pipette which was then attached to the container.

Delivery of the semen could only be made slowly through the narrow pipette; throughout the operation the sound was pressed home against the cervical os. After withdrawal of the pipette the loss of semen at the vulva was virtually nil from sows inseminated into the uterus. Visible losses after cervical and vaginal insemination were variable but were almost always noticeable after vaginal insemination. Leakage during cervical and vaginal insemination frequently occurred into the cavity of the sound; the use of a sound drilled to give the pipette a closer fit might have prevented some of this loss.

*Recovery and Examination of Ova*

Sows were slaughtered 24 to 72 hours after

insemination. Ova were recovered by flushing the fallopian tubes and uterus with saline and were examined without fixation for evidence of cleavage. Uncleaved ova were also examined by phase-contrast microscopy after fixation with acetic-alcohol (Hancock, 1958b). Cleaved ova and pronucleate ova were regarded as fertilised. Counts were made of the number of spermatozoa on the zona pellucida of fixed ova. With the preparations used, most of the spermatozoa lie in two focal planes, one above the ovum and one below and counting is easy except where the spermatozoa are very numerous. Ova were divided into 5 classes according to the number of spermatozoa present on the zona pellucida :—

1. None
2. 1 to 10
3. 11 to 50
4. 51 to 200
5. Innumerable

"Conception rate" in this paper is used to indicate the percentage of all sows from which some fertilised ova were recovered.

A sow from which some fertilised ova were recovered is called "fertile" and is said to have "conceived."

The fertilisation frequency is the percentage of recovered ova found to have been fertilised.

## Results

All of 18 sows mated naturally conceived and the over-all fertilisation frequency for the 6 boars tested ranged from 86.5 to 100 (mean frequency, 95.9). The data gave no evidence of any significant difference in fertility between boars.

Of a total of 51 artificial inseminations, 27 were uterine, 10 cervical and 14 vaginal. Of the 27 sows inseminated into the uterus, 26 had conceived. Five of 10 cervical inseminations and 8 of 15 vaginal inseminations resulted in conception.

The fertilisation frequencies for inseminations at the 3 sites are for all sows 84.1, 52.3 and 33.3, and for fertile sows 87.7, 100 and 59.8. The mean conception rate for all sows inseminated was 76.5 ; the mean fertilisation frequencies were 64.6 (all sows) and 84.1 (fertile sows).

Several factors may have affected the success of attempts at intra-uterine insemination. Only 6 of the 20 sows inseminated under restraint were uterine inseminations. Of 31 sows inseminated free of restraint, 21 were made into the uterus. Uterine insemination was successfully done in 7 of 10 unmated sows and of 20 of 40 mated sows. Of 22 sows inseminated on day O, 12 were uterine, 2 were cervical and 8 were vaginal ; for 29 sows inseminated on day 1, the figures are 15 uterine, 8 cervical and 6 vaginal.

Because all vasectomised matings were made (to define the onset of heat) on day O, any effect of the interval between sterile mating and insemination is contained in the data for inseminations made on day O and day 1 omitting the results for unmated sows. Of 17 inseminations of mated sows on day O, 8 were uterine, 2 were cervical and 7 were vaginal. Of 24 inseminations on day 1 to mated sows (mated on day O), 12 were uterine, 7 were cervical and 5 were vaginal.

There is no conclusive evidence of an effect of any of the above factors on the success of insemination.

For inseminations with method A most of the variation in conception rate was clearly due to variation in the site reached for insemination and there is some evidence that the site reached is affected by the sow.

Table I shows the results got with method A on a single contemporary group of 25 sows of 4 inbred lines in which all inseminations were made on day 1 and were made without restraint except for 2 sows. The data for this group are examined separately because it is believed that no gain in technical proficiency was made in this period, technique was standardised and this sample of sows gives the best available picture of the efficiency of the method. Nine of 11 "P" line sows were inseminated into the uterus. Of 8 inseminations of "Y" line sows,

TABLE I

THE SITE REACHED FOR INSEMINATION (UTERUS, CERVIX, VAGINA) AND THE RESULTS OF INSEMINATION IN 25 SOWS (CONTEMPORARIES) OF FOUR INBRED LINES (P, Y, A and S) INSEMINATED BY METHOD A. OPEN FIGURES REFER TO THE NUMBER OF SOWS INSEMINATED (TOTAL) AND THE NUMBER (n) AND PERCENTAGE WHICH CONCEIVED. FIGURES IN BRACKETS REFER TO THE NUMBER OF OVA RECOVERED (TOTAL) AND NUMBER (n) AND PERCENTAGE FERTILISED

| Sow line | Uterus | | | Cervix | | | Vagina | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total | Conceived | | Total | Conceived | | Total | Conceived | |
| | | n | Per cent. | | n | Per cent. | | n | Per cent. |
| P | 9 | 9 | 100 | 2 | 2 | 100 | 0 | — | — |
| | (127) | (123) | (96·8) | (27) | (27) | (100) | — | — | — |
| Y | 5 | 4 | 80 | 3 | 0 | 0 | 0 | — | — |
| | (71) | (52) | (73·2) | (27) | (0) | (0) | — | — | — |
| A | 3 | 3 | 100 | 0 | — | — | 0 | — | — |
| | (31) | (29) | (93·5) | — | — | — | — | — | — |
| S | 0 | — | — | 0 | — | — | 3 | 0 | 0 |
| | — | — | — | — | — | — | (34) | (0) | (0) |
| All | 17 | 16 | 94·1 | 5 | 2 | 40 | 3 | 0 | 0 |
| | (229) | (204) | (89·1) | (54) | (27) | (50) | (34) | (0) | (0) |

TABLE II

THE NUMBER OF SPERMATOZOA IN THE ZONA PELLUCIDA OF ALL OVA AND OF CLEAVED OVA* FROM MATED SOWS (M), FROM SOWS INSEMINATED BY METHOD A INTO THE UTERUS, CERVIX AND VAGINA, AND FROM SOWS INSEMINATED BY METHOD B

| Mating method | | All ova | | | | | | Cleaved ova | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of sows | Total | 0 | 1–10 | 11–50 | 51–200 | Innumerable | Number of sows | Total | 0 | 1–10 | 11–50 | 51–200 | Innumerable |
| M | | 14 | 133 | 13 | 26 | 31 | 39 | 24 | 12 | 107 | 6 | 13 | 26 | 38 | 24 |
| A | Uterus | 14 | 176 | 12 | 38 | 45 | 53 | 28 | 14 | 172 | 11 | 36 | 45 | 52 | 28 |
| A | Cervix | 4 | 43 | 24 | 7 | 6 | 6 | 0 | 1 | 16 | 3 | 1 | 6 | 6 | 0 |
| A | Vagina | 5 | 47 | 43 | 0 | 4 | 0 | 0 | 1 | 5 | 1 | 0 | 4 | 0 | 0 |
| B | | 8 | 74 | 22 | 13 | 16 | 16 | 7 | 7 | 47 | 5 | 5 | 14 | 16 | 0 |

* Pronucleate ova are not included.

5 were intra-uterine and 3 were intra-cervical; two of these 3 were sows inseminated under restraint. All of 3 "A" line sows were successfully inseminated into the uterus but in 3 "S" line sows (littermates) it was difficult to pass the vaginal sound as far as the cervical os and in all it was doubted at the time of insemination that the external os had been reached.

In view of failure in a number of cases to inseminate into the uterus by method A some inseminations were made with method B. It was noticeable that sows resented the introduction of the cuffed pipette more than they did the sound used to carry the pipette in method A. Cleaning and sterilisation of equipment used in method B were also more difficult. However, examination of the data for all inseminations by the two methods shows little difference between the two methods whether judged by the number of sows which conceived (method A, 38 of 51; method B, 10 of 12) by the percentage of all ova fertilised (method A, 64.6; method B, 59.2) or by the percentage of fertilised ova from fertile sows method A, 84.1; method B, 71.4).

In a small experiment planned to compare the two methods more precisely, sows, as they became available, were allotted to one of two groups for insemination by method A or method B. All of 9 sows inseminated with method A conceived and the fertilisation frequency was uniformly high (95.4). Of 9 sows inseminated by method B, 7 had conceived; the fertilisation frequency for the 7 fertile sows was 73.1; for all sows it was 60.1.

Table II summarises the results of counts of the number of spermatozoa on ova from mated and inseminated sows. There is evidence here that the numbers of spermatozoa found on recovered ova are not less on ova from sows inseminated successfully into the uterus than on ova from sows mated naturally.

## Discussion

There are 3 questions to be answered as regards the intra-uterine insemination of sows.

1. Is intra-uterine insemination more efficient than insemination at other sites?

2. In what proportion of sows can it be done?

3. How does it compare in efficiency with other techniques?

The answer to the first question is clearly that, with the technique used here, insemination of sows into the uterus is more efficient than insemination into the cervix or vagina; in fact the fertility of insemination made into the uterus is of the order of the fertility of natural mating.

The answer to the second question is more difficult. It is shown here that success in uterine insemination is affected by a number of factors.

In these investigations there is no conclusive evidence of any effect of restraint of sow, of previous mating or of the day of insemination on the success of insemination. There is a hint however that inseminating mated sows and also sows under restraint on the second day of heat may have prejudiced the success of uterine insemination. There is therefore no reason to believe that uterine insemination would be less successful under field conditions than here. Evidence of an effect of sow is also inconclusive but it is clearly possible that success with this method might vary between different samples of pigs. The operation demands a certain amount of technical proficiency but certainly not more (although probably not much less) than is needed for the insemination of cattle.

The evidence as regards the answer to the third question is again scanty but it seems clear that under the conditions of this investigation the method is at least as efficient as the method devised by Aamdal and in other respects it is potentially better. In this connexion it is of interest that Holt (1959) has reported that the proportion of sows farrowing to artificial insemination declined with increasing pipette calibre; his best results were from inseminations made with a pipette of the calibre used here (3 mm.). These pipettes have the advantage that they can be produced sufficiently cheaply for them to be discarded after use; this eliminates the need for cleaning and sterilisation and provides greater insurance against the transmission of disease although the need for efficient sterilisation of semen containers and vaginal sound remains.

The observation that visible loss of semen is much less evident after intra-uterine insemination than after insemination at other sites is regarded as evidence that intra-uterine insemination is likely to be more efficient. It may well be that the use of intra-uterine insemination will permit a considerable reduction in the size of the insemination dose both as regards numbers of spermatozoa and volume of inseminate. If this is true the difference in efficiency

# Experimental *Trypanosoma vivax* Infection in the Horse

BY

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AND

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*SUMMARY.*—1. *An experimental fly-transmitted infection with* Trypanosoma vivax *in a horse is described giving details of clinical signs, haematology, and clinical chemical examinations.*

2. *The infection was characterised by a series of parasitaemic peaks accompanied by pyrexia, polypnea, tachycardia, oedema of the legs, hydrocele, and muscular tremors.*

*Urticarial swellings and icterus appeared early and occurred throughout the infection. As the disease progressed appetite diminished, severe anaemia developed, and the animal became dull and weak with marked loss of condition and walked with a swaying gait.*

3. *Clinical chemical studies revealed elevated serum bilirubin, gamma-globulin, plasma specific gravity readings, and a positive formol gel test during the course of the infection.*

4. *The infection was successfully cured with ethidium bromide injected intramuscularly, as a* 2 *per cent. solution, at a dose rate of* 1 *mg. per kg. bodyweight.*

A NUMBER of observers of clinical trypanosomiasis in the horse have indicated that *Trypanosoma vivax* infection is a relatively innocuous disease.

Bouffard (1912) states that *T. cazalboui* (=*T. vivax*) in French West Africa, as a rule, causes a chronic disease in horses which may last from 2 to 4 years without treatment. Macfie (1913) working in Northern Nigeria refers to it as a mild disease from which 14 out of 15 infected horses recovered. Curson (1928) is of the opinion that horses are comparatively tolerant to infection with this parasite. A strain of *T. vivax* from Ruanda was described by Rodhain, van Goidsenhoven, and van Hoof (1941) as having little pathogenicity for horses. Hornby (1952) reported that although he had many fly-struck horses to treat during the German East African campaign, pure *T. vivax* infections were rare, and these he recorded as being of a chronic character. Henning (1956) also states that horses are comparatively tolerant to infection with this protozoon.

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**Pig Insemination Technique**—*Concluded.*

between intra-uterine insemination and other methods might well be found to be even more clearly in favour of the uterine method when the comparison is made with a lower semen dosage than that used here. More work is needed to confirm or refute this suggestion.

The low fertility of inseminations made into the cervix and vagina presents a special problem and it is clear that something will need to be done to increase the chances of fertilisation in those cases where attempts to inseminate into the uterus prove unsuccessful. It might be practicable to try to combine the advantages of the intra-uterine method with Niwa's method. With his method the insemination pipette is formed of stout rubber and has a pear-shaped nozzle which is said to engage in the cervical folds. There seems no reason why the sound which carries the flexible pipette should not be similarly shaped and used where intra-uterine insemination is impracticable in the same way as the Japanese pipette. It would be important in these circumstances that the inner pipette should form a snug fit in the end of the sound to prevent leakage back *via* the cavity of the sound.

Quite apart from its use for commercial insemination of pigs it seems likely that because of the uniformity of results got with intra-uterine insemination the method is the method of choice for certain experimental investigations on factors affecting the fertility of pigs. Failure to inseminate some sows into the uterus would not be serious because these could be discarded from the experiment at the time of insemination. This loss could well be offset by the gain in uniformity.

*Acknowledgments.*—It is a pleasure to record my thanks to A. F. Holt and D. H. L. Madden for samples of equipment and to them and to L. E. A. Rowson, V. J. A. Manton and C. Polge for helpful discussions.

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In an earlier paper (Stephen & Mackenzie, 1958) we reported a case of *T. vivax* infection in a mare. In this case the infection was treated on the same day that trypanosomes appeared in the peripheral blood. The animal showed considerable improvement following treatment, particularly of the blood picture; and we attributed this to the fact that the trypanosome infection had been cured.

An account is now given of the clinical signs and clinical pathological effects caused by a recently isolated, cyclically passaged, strain of *T. vivax* in a horse.

### Materials and Methods

*Subject.* The horse used in this experiment was an aged, brown and white, Arab stallion. The animal was in excellent physical condition, and had no history of illness during the 3 years preceding the experiment. He could not be ridden because of a chronic sesamoiditis and was due for euthanasia. Throughout the experiment he was kept in a loose-box and feed was offered at the rate of 6 lb. of guinea corn (*Sorghum* spp.), and about 15 lb. of Acha straw (*Digitaria exilis*) per day. The horse was examined twice daily, morning and evening, and a record was kept of clinical signs, including temperature, pulse, respirations, faeces, urine, mucous membranes and appetite.

*Blood.* The following examinations were made on the blood at weekly intervals before infection, twice a week during infection and until just after treatment, and thereafter weekly or fortnightly for 24 weeks :—

(a) Red Cell Count—Thoma haemocytometer.
(b) Packed Cell Volume—Centrifuged Wintrobe Haematocrit tubes at 3,000 r.p.m. for 1 hour. Radius = 5.5 inches.
(c) Haemoglobin—1. Sahli haemoglobinometer.
2. Medical Research Council, Grey-Wedge Photometer.
(d) Serum Gamma Globulin—Kunkel (1947) turbidimetric technique, standardised against saline solutions of Armour purified gamma globulin.
(e) Serum Bilirubin—quantitative estimation by the method of King and Coxon (1950), using the standard of Patterson, Swale and Maggs (1952).
(f) Serum Non-Protein Nitrogen—estimation by Kjeldahl digestion and nesslerisation (King, 1946).
(g) Plasma Specific Gravity—copper sulphate method (Phillips, Van Slyke, Dole, Emerson, Hamilton & Archibald, 1945).
(h) Formol Gel Test—one drop of 40 per cent. formaldehyde solution added to 1 ml. serum (Napier, 1921). The test was read after 24 hours at approximately 25° C. because positive reactions develop slowly in cattle and horse serum. Three grades of reaction were differentiated, "O" = liquid, "1" = solid and clear, and "+" = solid and opaque.

Wet blood films were examined for trypanosomes once a week for 5 weeks prior to challenging with tsetse-flies. Thereafter films were examined daily until one month after treatment and subsequently to coincide with follow-up blood examinations. One hundred microscopic fields were examined on each occasion.

*Challenge.* One hundred and fifty laboratory-bred *Glossina palpalis* were obtained, in batches of 50, from the Entomology Section of the West African Institute Trypanosomiasis Research at Kaduna. These flies were fed on a sheep that was infected by wild-caught *G. morsitans* and had shown only *T. vivax* infection in its peripheral blood. The flies were maintained on the infected sheep for at least 13 days before challenging the horse.

The horse was challenged on 3 separate occasions at intervals of one week. Each challenge consisted of permitting approximately 35 flies, contained in Bruce boxes, to feed on 3 consecutive days. On the day after the last feed, each batch of flies was dissected to determine the mature infection rate. One hundred of the trypanosomes causing the infection were measured by bench projection using a Leitz Ortholux microscope.

*Treatment.* Previously it had been decided that the horse would remain infected until it was clinically ill. It was then to be treated with "ethidium

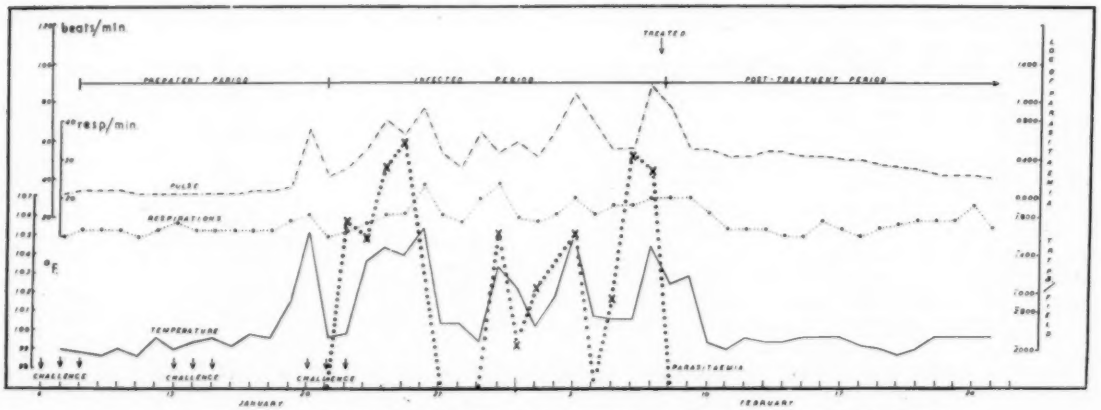


FIG. 1.—The effects of *T. vivax* infection on temperature, pulse, and respiration in a horse.

bromide" (2 : 7 diamino-10-ethyl-9-phenyl-phenanthridinium bromide) in the same manner as the mare (Stephen & Mackenzie, 1958).

**Results**

*Clinical Signs.* Trypanosomes were found in the peripheral blood 16 days after the first fly challenge. During the 16 days that the infection was permitted to run, there were 4 distinct peaks of parasitaemia, followed by either absence of trypanosomes or a low level of parasitaemia. These crises were accompanied by pyrexia and fluctuations in the pulse and respiration rate (Fig. 1). There was a distinct temperature peak lasting for 2 days and occurring 3 days before trypanosomes were found in the peripheral blood.

Urticarial swellings appeared in the saddle area of the horse's back 10 days after the first fly challenge, *i.e.* on the day following the completion of the second fly challenge. These swellings recurred periodically during the infection, coinciding with the interval between parasitaemic and temperature crises. On these latter occasions they appeared on the face, neck and body.

The conjunctivae were distinctly congested 3 days before the appearance of trypanosomes; and a definite icterus was present on the day that they were seen in wet films. The icterus was pronounced until 2 days after treatment, although the conjunctivae and gums became increasingly paler as the disease progressed.

The scrotal sac was distended, more or less, throughout the period of infection, but the greatest distension coincided with the peaks of parasitaemia and temperature. Return to normal was rapid following treatment.

Oedema of the legs was pronounced, beginning with the onset of parasitaemia. This commenced at the fetlock and gradually rose to the level of the carpus and hock. Once again however, the swelling was most clearly evident during the peak periods of parasitaemia.

The horse developed muscle tremors 3 days before trypanosomes appeared. This was first noticeable in the triceps muscle, but later it also affected the latissimus dorsi and other superficial muscles of the thorax, abdomen and neck. As the animal became progressively weaker a distinct knee tremor developed.

By the fifth day of infection the horse walked with a swaying gait and this became progressively worse, until at 7 days the animal was having difficulty rising, and when it did so it had great difficulty maintaining its balance.

Appetite varied between good and poor throughout most of the period of infection, but towards the fifteenth day it had definitely become poor. During the week before trypanosomes appeared in the peripheral blood the horse lost 33 lb. in weight and a further 42 lb. during the period of infection.

On the sixteenth day of the infection the horse was walked to the balance for weighing prior to treatment. It was then very weak and walked with difficulty, swaying and dragging its feet. When standing it placed the legs wide apart to avoid falling down. The walk to the balance, a distance of 30 yards, caused great fatigue, polypnoea and a pulse rate of 120 beats per minute.

*Treatment.* After the infection had progressed for 16 days, it was decided to treat the horse. Ethidium bromide at a dose rate of 1.0 mg. per kg. bodyweight, in a 2 per cent. solution (total volume= 15.8 ml.) was injected intramuscularly into the gluteal muscles, one half of the total dose in each side.

Within an hour of dosing the horse was down and could not be induced to rise. He showed signs of apprehension and continually raised the head to the flank and lowered it to the floor. Body temperature was 103.8° F., pulse 100 per minute and respirations 60 per minute; and there was copious perspiration in the inguinal region. One hour later, *i.e.* 2 hours after dosing, the horse was on its feet and eating. Five hours later he looked qute well, but the temperature had risen to 105° F.

The temperature and respiration rate dropped to pre-infection levels by the fourth day after treatment, but the pulse rate remained higher until about a month after treatment.

No trypanosomes could be found in the peripheral blood 24 hours after dosing and the animal has remained free from infection for 10 months.

The drug was well tolerated at the site of injection; a slight swelling only occurred and this soon disappeared.

*Haematology.* The erythrocyte count, packed cell volume per cent. and haemoglobin values fell considerably during the prepatent period, but the most rapid decline came after trypanosomes appeared in the peripheral blood (see Fig. 2). All of these determinations rapidly rose to pre-infection levels following treatment.

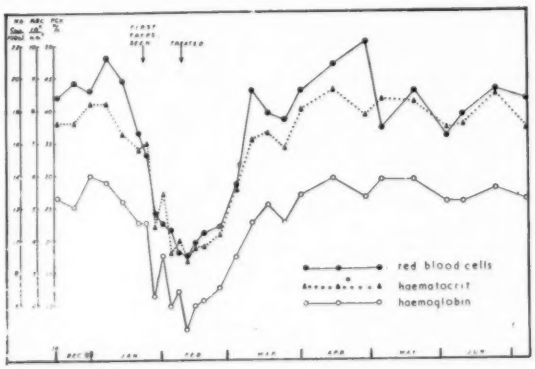


FIG. 2.—The blood picture in a horse before and during infection with *T. vivax* and its return to pre-infection levels after treatment with ethidium bromide.

*Clinical Chemistry.* Bilirubin appeared in the serum as soon as trypanosomes were detected in the peripheral blood (see Fig. 3). Prior to infection, examination for this pigment was negative on 6 occasions, but after treatment, although the level dropped considerably, negative reactions were seen

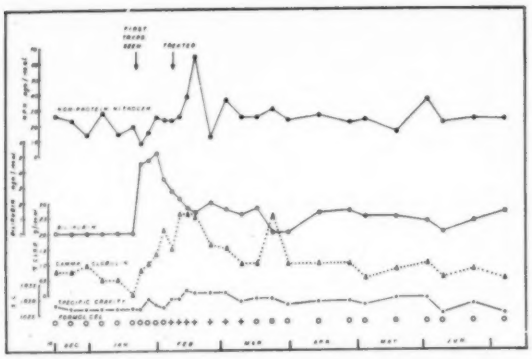

FIG. 3.—The effects of *T. vivax* and treatment with ethidium bromide on some clinical chemical values in the blood of a horse.

only in 3 out of 20 examinations performed during the 6 month follow-up period.

The non-protein nitrogen reading rose sharply 10 days after treatment.

The formol-gel test became positive (reaction= " 1 ") at the end of the infection period and remained so for one month after treatment.

The gamma-globulin level rose soon after infection and continued above pre-infection levels until a month after treatment. The plasma specific gravity rose correspondingly during this period.

*Fly Challenge.* The fly challenge data is summarised in Table I from which it will be seen that the *mature* (hypopharyngeal) infections ranged from 70.6 per cent. to 91.4 per cent. *T. vivax* infections in tsetse-fly are considered to be immature if only the labrum contains trypanosomes and mature if the hypopharynx is infected.

TABLE I
FLY CHALLENGE DATA—*G. palpalis*

| Fly Batch Number | Period fed on infected sheep prior to challenging horse | Number of flies dissected | Percent mature *T. vivax* infections (Hypopharynx) |
|---|---|---|---|
| 1 | 15 days | 35 | 91.4 |
| 2 | 13 days | 36 | 83.3 |
| 3 | 18 days | 34 | 70.6 |

*Trypanosome.* The mean length of the organism was 21.11 $\mu$ (S.E. of Mean = $\pm$0.171); the distribution was found not to deviate significantly from normal (P between 0.05 and 0.02).

## Discussion

There is no doubt in our minds that *T. vivax*, or at least the organism causing the infection in this case, is pathogenic for the horse.

The clinical disease as we have observed it consists of a series of parasitaemic crises, and these are accompanied by pyrexia (the highest afternoon temperature recorded was 107.4° F.), polypnoea, tachycardia, oedema of the legs below the carpus and hock, hydrocele and muscular tremors. Urticarial swellings may appear on any part of the body during the prepatent period and between crises throughout the infection. Icterus of the conjunctivae and gums appears early in the infection, and, persists until treatment is given. As the disease progresses, the appetite diminishes, a severe anaemia develops and the animal becomes dull and weak, with marked loss of condition, and walks with a swaying gait.

The prodromal temperature peak occurring 2 days before trypanosomes appeared in the peripheral blood (see Fig. 1) possibly indicates the end of the incubation period for the metacyclic trypanosomes (the site of whose development is unknown) although they were not detected in the wet blood films.

The swelling of the scrotal sac undoubtedly indicates the presence of oedema in the cavity of the tunica vaginalis rather than an orchitis, because the swelling decreased considerably between temperature peaks and returned to normal 3 days after treatment.

Icterus of the conjunctivae was visible only when the bilirubin levels were above 2.0 mg. per 100 ml. of serum. It is also interesting to note that examinations for bilirubin before infection were all negative, but after treatment negative reactions were seen only 3 times in 20 examinations over a period of 6 months.

Ramsey (1946) determined the bilirubin concentration in the blood plasma obtained from a large number of horses. The mean concentration was 1.57 mg. per 100 ml. The standard deviation was $\pm$0.86 mg. per 100 ml. The subject in this study however appeared to develop a bilirubinaemia only after a pathological stimulus.

The swaying gait seen during the infection was thought to be due to muscular weakness rather than to a locomotor ataxia caused by pathological changes in the central nervous system.

The anaemia of trypanosomiasis is not well understood, but in the case of the horse it appears to be due to mass destruction of erythrocytes rather than to a direct anti-erythropoietic action on the bone marrow. It may be seen from Fig. 3 that the serum bilirubin was high during the period that the red cell count was dropping rapidly, but became less as the total number of erythrocytes declined; and further the erythrocyte count required only one month to return to pre-infection levels following treatment.

Mean corpuscular haemoglobin concentration values showed very little variation from pre-infection levels, indicating that the cell haemoglobin content did not alter greatly. Mean corpuscular volume figures, however, indicated that periodic attempts were made to combat the anaemia of the infection by a macrocytosis.

As mentioned above under materials and methods, the haemoglobin content of the blood was estimated by the Sahli and M.R.C. Grey-Wedge photometer techniques. In our hands, the readings with the Sahli haemoglobinometer have always been higher than those with the Grey-Wedge instrument. The average difference between them was 2.43 gm. per 100 ml., range 1.2 to 4.1 gm. per 100 ml. of blood. The readings with both instruments agreed most closely at the lower haemoglobin values.

(*Concluded at foot of page overleaf*)

# Swelling of the Infraorbital Sinus in Poultry Caused by an Infection with *Mycobacterium tuberculosis*

BY

J. M. S. LUCAS and J. M. INGLIS

*SUMMARY.—Two cases of avian tuberculosis in which a unilateral swelling of the infraorbital sinus was an obvious symptom are described.*

## Introduction

The symptoms of tuberculosis (*Mycobacterium tuberculosis* avian type) in adult hens are generally indefinite, and are associated with wasting and loss of condition, though lameness may be noted in some cases (1948). There does not appear to be any previous record of lesions involving the infraorbital sinus in this disease.

*SUBJECT.* Two Rhode Island Red × Light Sussex hens in the second year of lay.

*Owners' complaint.* The two birds, members of a flock of 17, had developed swollen eyes.

*History*

The birds were from a domestic, self-contained flock of 17 birds. The incontact birds were apparently healthy, though one was lame and required hand feeding. A few months previously one bird had been killed as it had "gone-light."

**Experimental *T. vivax* Infection in the Horse**—*Concluded.*

The conjunctivae returned to a healthy pink colour one week after treatment, but it is interesting to note that the pulse rate did not slow down to pre-infection rates until about a month after treatment. This was probably due to the anaemia caused by the infection.

The rapid rise in gamma-globulin following the appearance of trypanosomes indicates an active immunity response on the part of the horse, but this did not seem to affect the virulent course of the infection appreciably. The plasma specific gravity readings closely paralleled the gamma-globulin determinations, and it is probable that the unit weight of the plasma varied directly as the content of this protein. The copper sulphate technique, although it reveals variations in specific gravity of 0.001, is not a particularly sensitive reflection of the serum gamma-globulin however.

The formol-gel test has been used for the diagnosis of Leishmaniasis in man, where jellification with opacity within 20 minutes constitutes a positive reaction; although Napier (1923), who developed the test, suggested that readings be made at various times up to 24 hours. The reactions seen with the horse's serum were slow to develop, and a positive test consisted of a clear gel at 24 hours. Since this test became positive only when the horse was seriously ill, it is doubtful whether it has any value as a diagnostic aid in this disease.

The transient rise in the non-protein nitrogen 10 days after treatment may possibly indicate that there was some kidney damage due to the drug, but no permanent damage appeared to have been caused.

The trypanosome causing this infection was somewhat shorter than most strains of *T. vivax* reported by Fairbairn (1953) and slightly longer than the organism causing the infection in the mare (Stephen & Mackenzie, 1958).

The systemic reaction that occurred one hour after dosing with ethidium bromide was rather alarming, and we would suggest therefore that this drug be administered to infected horses in a divided dose of say one half, followed by the other half after a 6-hour interval. It is not known whether these effects were due to the drug *per se*, or to an endotoxin liberated by the large numbers of trypanosomes killed by the drug.

It is proposed to investigate this phenomenon further.

*Acknowledgments.* The writers are indebted to Dr. D. G. Godfrey, Protozoology Section, W.A.I.T.R., Vom, for measuring the trypanosomes and for the statistical analysis of these measurements. Thanks are also due to Mr. H. A. Orhue, Veterinary Assistant to the Clinic of the Veterinary School, Federal Veterinary Research Laboratories, Vom, for his assistance during this experiment.

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1a

1b

FIG. 1.—Infraorbital swelling in infected hens.

Appetite and egg production were considered by the owner to be satisfactory. The swellings complained of had developed over a few days.

*Symptoms*

The birds appeared to be in very good condition. Below the left eye in each bird there was soft swelling almost the size of a golf ball. In one of the birds the conjunctival sac had become everted due to pressure from the swelling (see Fig. 1). The eyes were clear and apparently unaffected and no nasal discharge was apparent.

*Examination and Treatment*

On examination of the conjunctiva, there was found in each of the affected birds, attached to the conjunctiva, or in the conjunctival sac of the affected eye, a piece of necrotic tissue, which was similar in size, colour and texture to a piece of boiled maize; this was removed exposing granulation tissue. The owner was advised to bathe the affected eye in saline.

After two days the swellings were definitely larger and the birds were returned for examination. Flakes of yellowish necrotic tissue were found attached to the conjunctiva in each of the birds. Smears of this material stained using the Ziehl-Neelsen technique were found to contain numerous acid-fast bacilli indistinguishable from *Mycobacterium tuberculosis*. The birds were destroyed.

Post-mortem *Examination*

The carcases were well covered with flesh, and the amount of peritoneal fat seemed to be normal. A few yellowish white caseous nodules were present in the livers of both birds and the spleen of one. The left infraorbital sinus of both birds was distended by a yellow jelly-like material, in which there were numerous acid-fast organisms.

Inoculation of Lowenstein-Jensen medium with material from the caseous nodules in the liver and spleen and material from the infraorbital sinus produced growth typical of *Mycobacterium tuberculosis* avian type.

*Seauclae*

A tuberculin test was carried out on the remaining 15 birds in the flock using Ministry of Agriculture Tuberculin (Avian. P.P.D.) injected into the right wattle. All but 5 developed reactions to the test.

The case is thought worth recording as swelling of the infraorbital sinus appears to be an unusual symptom of tuberculosis infection in hens.

**Reference**

"Handbook on Poultry Diseases," 2nd edit., N.V.M.A., 1948.

## THE SHARE-JONES LECTURE

The first Share-Jones Lecture will be given on Thursday, October 1st, 1959, at 5 p.m., at the Royal Veterinary College, when Mr. H. V. Hughes will speak on "The Skin of the Dog."

# News and Comment

## "THE VETERINARY RECORD"

The dispute in the printing industry having terminated on August 6th, we are now able to return to normal production. Readers will notice that page and issue numbers have been continued from the last published issue, that of June 20th, so that when volumes are bound the last weekly issue will be numbered 45 and not 52. The three fortnightly emergency numbers produced by duplication will be separately indexed so as to avoid confusion.

## UFAW SUMMER SCHOOL

The UFAW Summer School will be held at Knuston Hall, Irchester, Wellingborough, Northamptonshire, on Friday, 18th, to Friday, 25th September, 1959. The programme will include visits to the School of Veterinary Medicine, Cambridge; the Nutritional Research Unit, Huntingdon; and Whipsnade Zoo.

Talks will be given on the following subjects: "The Research for a Humane Rodenticide"; "The Use of Animals in Nutritional Research"; "The Work of the International Committee on Laboratory Animals"; "Animals in English Law"; "The Transport and Marketing of Animals"; "Anaesthesia for Laboratory Animals"; "Transport of Laboratory Animals"; etc.

Fees for Seniors will be £6 10s. and the special reduced fee for students will be £3 10s. These fees include full board and all excursions. A few bursaries are available and full details of these will be given on request.

Enquiries and applications should be made to: Miss Williams, UFAW, 7a, Lamb's Conduit Passage, London, W.C.1. Telephone: CHAncery 9221.

## INTERNATIONAL POULTRY AWARD

The Newman Trust International Poultry Award Committee in the United Kingdom has decided that the 1959 Award shall be made to Professor F. W. Hill, Professor of poultry husbandry, Cornell University, Ithaca, New York. There were submissions this year from the Union of Soviet Socialist Republics, Canada, and the United States of America. The Award was instituted some years ago by Poultry Association of Great Britain and has been won 7 times by U.S.A. research workers and 3 times by United Kingdom workers. It consists of a medal and £50 sterling, and is given for what, in the opinion of the Award Committee, is the most important contribution to poultry husbandry research work published in the previous year.

The Award was made to Professor Hill for his work in poultry nutrition published in 1958.

## THE REGISTER OF VETERINARY SURGEONS

The names of the undermentioned gentlemen were entered in the Commonwealth List on July 22nd, 1959:—

Azzie, Maurice Anthony Joseph, B.V.SC. (PRETORIA), 24, Jonkershoek Road, Hill Extension, Johannesburg.

Widdows, Frederick Arthur, B.V.SC. (SYDNEY), 21, Kareela Road, Cremorne, N.S.W., Australia.

Mr. David Flatt, D.V.M. (ONTARIO), c/o Mr. E. E. Burgis, 41, Wayside Avenue, Bushey Heath, Herts., was entered on July 10th.

The name of Gabriel Henri Audebert, DR.MED.VET. (ALFORT), 4, Place de la Tranchée, St. Symphorien, Indre et Loire, France, was restored to the Register of Veterinary Surgeons on August 10th, 1959, in accordance with the provisions of Sec. 25 (4) of the Veterinary Surgeons Act, 1948, and Statutory Instrument No. 1729 of 1957.

## THE COLONIAL VETERINARY SERVICE

**May List**

C. N. Chappel, M.R.C.V.S., Veterinary Officer, Dominica, Windward Islands, has been transferred to Jamaica, as Veterinary Officer.

*New appointments.* E. A. Holt, M.R.C.V.S., Supernumerary Veterinary Officer, Windward Islands; stationed at Dominica. D. I. McKinlay, M.R.C.V.S., Veterinary Officer, Northern Rhodesia.

**June List**

J. G. Black, D.V.M. (TOR.), M.S.A. (TOR.), D.V.P.H., D.V.S.M., Livestock Improvement Officer, Kenya, has become Principal Livestock Improvement Officer, Kenya.

*New appointments.* A. R. Ferguson, M.R.C.V.S., Veterinary Officer, Gambia. S. R. Rippon, M.R.C.V.S., Veterinary Officer, Somaliland Protectorate (contract).

## UNIVERSITY NEWS

**Edinburgh**

Past and present students of the "Dick," and others connected with that School, will learn with much satisfaction that, as the only veterinary department to be invited to take part in the scientific exhibition at the meeting of the British Medical Association and Canadian Medical Association, the Department of Anatomy was awarded a certificate of merit. The exhibit, which was under the charge of Mr. T. Grahame, had for subject "The Kidney in the Animal World," and was described thus in the catalogue:—

"The exhibition shows the injected vascular systems and ureters of a variety of animals. Some have been injected with celluloid and others with vinylite. The main purpose is a comparative study of the distribution of the vessels and the character of the pelves and calyces."

The certificate of merit was given for scientific value and originality; and as there were 80 medical exhibits and only one veterinary, the Department of Anatomy at the "Dick" are cordially to be congratulated.

The Diploma in Veterinary State Medicine has

been awarded to Mr. A. H. Watson, M.R.C.V.S., of Reading, Berks.

**Giessen**

In the course of the exchange of British and German professors organised by the British Council, the Faculty of Veterinary Medicine of the Justus Liebig University of Giessen invited Professor J. G. Wright, Dean of the Faculty of Veterinary Science, University of Liverpool, to deliver two guest lectures. On July 14th Professor Wright spoke on "The Place of Caesarean Section in Bovine Obstetrics," and on July 15th on "The Present Status of Anaesthesia in Horses and Cattle." Both lectures were received with great interest and appreciation by the German audience. Professor Wright was the guest of the Faculty of Veterinary Medicine during his stay in Giessen. In a special session he described to the members of the Faculty the education of veterinary students in the University of Liverpool. A discussion followed.

On the occasion of the 353rd anniversary celebration of the Justus Liebig-University, held on July 1st, the Faculty of Veterinary Medicine conferred upon Dr. R. Daubney, C.M.G., the honorary degree of Doctor in Veterinary Medicine.

Dr. Daubney was the guest of the Faculty, and delivered a lecture entitled "Near Eastern Equine Encephalomyelitis," in which he described recent researches carried out in collaboration with Dr. E. A. Mahlau.

**Liverpool**

*Faculty of Veterinary Science, Degree of B.V.Sc., Second Examination, Part III (Animal Management)*

Bardgett, P. L.; Bradley, W. A.; Cameron, A.; Cope, B. T.; Curran, M. K.; Fabry, A.; Gooderham, K. R.; Hall, Elizabeth; Hallows, B. J.; Hammonds, Patricia; Hardy, Elizabeth A.; Hilton, D. W.; Jones, R. T.; Jones, T. O.; Llewellyn, C. A.; Mohammed, I.; Moore, J. C.; Mudd, A. J.; Newcomb, R.; Pierrepoint, C. G.; Pulley, Norma M.; Rawson, B. C.; Riffkin, G. G.; Sopronfalvy, M. G.; Strong, M. G.; Summers, D.; Taylor, J. K.; Thompson, Hannah S.; Williams, H. G.

## EXPORT OF HORSES TO EUROPE: VETERINARY EXAMINATION FEE

Under the Diseases of Animals Act and Orders, horses being exported to European countries (subject to a number of exemptions, mainly for high-quality animals), are required to be examined at the port of embarkation by a veterinary inspector who must certify immediately before the animals are allowed to embark that they are fit to travel and to work without suffering. The fee for the service was originally fixed, in 1910, at 2s. 6d.; it was increased in 1919 to 5s., at which figure it has remained until now.

As from September 1st, 1959, the fee payable for the veterinary examination will be raised from 5s. to £1. The increase is required to make the service self-supporting at present-day costs.

This is the effect of an Order made jointly by the Minister of Agriculture, Fisheries and Food and the Secretary of State for Scotland, and copies of the Order, entitled the Exportation of Horses (Amendment) Order, 1959 (S.I. 1959 No. 1336), are obtainable from H.M. Stationery Office, price 2d. (by post 4d.).

## ARSENIC ON THE FARM

During the next few weeks, farmers are being warned by the Ministry of Agriculture to be particularly careful in their use of arsenical sprays to kill off haulm and weeds. These sprays are highly toxic to all animals, and if it is considered absolutely essential to use them, the greatest care must be taken.

The following safety recommendations have been put forward and should be suggested to clients: Before spraying, field fences must be made stock-proof and livestock moved from down-wind fields. Electric fences alone cannot be relied upon to keep out determined and inquisitive cattle. While spraying is in progress no unprotected person should be allowed in the field. Spray drift from any chemical spray is a nuisance but drift from an arsenical spray is especially dangerous, and it must be prevented by all possible means: spraying should be done only in calm weather. After spraying, gates of fields of treated crops should be securely fastened and warning notices erected. Stock should be kept out of treated fields until the treated haulm and weeds have been completely burned in small heaps throughout the field. If this is impossible at least 6 weeks should be allowed after spraying before letting stock into the field.

Used containers should be washed out, punctured to prevent further use, and disposed of safely, for example, by burying or burning.

## PERSONAL

**Engagement**

RENDALL—SMALL.—Graham F. Rendall, B.V.SC., M.R.C.V.S., of Beaminster, Dorset, to Margaret C. Small, of Ladymead, Langford, Nr. Bristol.

**Births**

CAMPBELL.—On July 22nd, 1959, at Keighley Victora Hospital, P.M.U., to Betty, wife of Donald Campbell, M.R.C.V.S., "Lydstep," Oakworth, Keighley, Yorks., a daughter, Elizabeth Grace.

COOK.—On June 9th, 1959, to Betty, wife of Terence F. Cook, M.R.C.V.S., of Raglan, New Zealand, a daughter, Joanna, sister for Philip, Penelope, and Alison.

HARRISON.—On July 9th, 1959, at Newmarket General Hospital to Josa, wife of Keith Harrison, M.R.C.V.S., a son, Simon Robert.

LE CHEMINANT.—On August 1st, 1959, in Guernsey, to Natica and David Le Cheminant, M.R.C.V.S., of Les Lâches, Cobo, a son, David Justin.

MACADAM.—On July 15th, 1959, at the Plateau Hospital, Jos, Northern Nigeria, to Pearl, wife of Ian Macadam, PH.D., M.R.C.V.S., B.SC., DIP.BACT., a son, Finlay Gregor, brother for Argyll Donald.

TAYLOR.—At Nurse Millers, Rochdale, on July 5th, 1959, to Shelagh (*née* Ford), wife of David C. Taylor, B.V.M.S., M.R.C.V.S., a daughter, Stephanie Jane Caroline.

## COMING EVENTS

### August

**30th to 5th September. 77th Annual Congress of the British Veterinary Association in Folkestone. (Official opening Monday, August 31st.)**

### September

9th (Wed.). Meeting of the Lincolnshire and District Division at the Berkeley Hotel, Doncaster Road, Scunthorpe, 2.15 p.m.

23rd (Wed.). General Meeting of the Lancashire Veterinary Association, Patton Arms, Warrington, 7.45 p.m.

24th (Thurs.). Meeting of the Supplementary Veterinary Register Division at 7, Mansfield Street, W.1, at 3 p.m. Committee meeting at 2 p.m.

25th (Fri.). Meeting of the North Wales Division at the Department of Agricultural Chemistry, Bangor, 2 p.m.

30th September, 1st and 2nd October (Wed., Thurs. and Fri.). Quarterly Meeting of Council, R.C.V.S.

## ADDRESSES OF DISEASE INFECTED PREMISES

The list given below indicates, first the county in which are situated the premises on which disease has been confirmed, followed by the postal address and date of outbreak.

### Anthrax

*Hunts.* Mill House, Mill Lane, Water Newton (Aug. 10).
*Lancs.* Baldwin Farm, Newton, Barrow-in-Furness (Aug. 10).
*Staffs.* Beechwood Farm, Onneley, Crewe (Aug. 6).

### Fowl Pest

*Beds.* Old Farm, Stanbridge, Leighton Buzzard (Aug. 1); Ivy Farm, Stanbridge, Leighton Buzzard (Aug. 10).
*Essex.* 22, West Street, Rochford (Aug. 2); Carnethy, Mill Lane, Danbury, Chelmsford (Aug. 10).
*Glouc.* Valley Farm, Staverton, Cheltenham (Aug. 5); Staverton Post Office, Staverton, Cheltenham (Aug. 6).
*Kent.* 71, Mill Lane, Herne Bay; Ambridge Poultry Farm, Longfield Avenue, Dartford (Aug. 4); 20a, Broomfield Road, Herne Bay; Ardmor Poultry Farm, School Lane, Herne Bay (Aug. 5); Belmont, The Aerodrome, Bekesbourne, Canterbury (Aug. 7).
*Rutland.* The Paddock, Cold Overton Road, Oakham; 151, Brook Road, Oakham (Aug. 1); 91, West Road, Oakham (Aug. 4).
*Surrey.* Classford Farm, Aldershot Road, Worplesden Guildford (Aug. 4).
*Yorks.* Low Fold Farm, Moss Carr, Keighley (Aug. 4); Long Lee Hall, Moss Carr, Keighley (Aug. 5).

### Swine Fever

*Ches.* Seven Stars Nurseries, Bridgemore, Nantwich; Woodside, Hatheston, Nantwich (Aug. 7); Shaw Fold Farm, Shaw Road, Heaton Moor, Stockport (Aug. 10).
*Devon.* Gulliford Gardens, Lyjpstone (Aug. 10).
*Essex.* Brickhouse Farm, Mundon, Chelmsford; Wyldberry, Fox Street, Ardleigh, Colchester (Aug. 4); York Road, West, Hawkwell, Hockley (Aug. 6); Llantrisant Oaks, Aveley Road, Rainham; Cobbs, Fossetts Lane, Fordham, Colchester (Aug. 10).
*Glouc.* Melton Farm, Lower Stone, Falfield (Aug. 7).
*Kent.* Kingsthorne Farm, Kingsnorth, Ashford (Aug. 4); Woodstock Farm, Hawkinge, Folkestone (Aug. 10).
*Lancs.* Ladyhouse Piggeries, Milnrow, Rochdale (Aug. 6); Tenement Farm, Thornley, Longridge, Preston; Middenstead Farm, Hoghton, Preston (Aug. 10).
*Mon.* The Elms, Sedbury, Chepstow (Aug. 5).
*Northants.* The Sycamores, Blakesley, Towcester (Aug. 4).
*Notts.* Wolds Farm, Cotgrave, Nottingham; Main Street, Willoughby-on-the-Wolds, Loughborough (Aug. 6).
*Salop.* 3, Uppington, Wellington (Aug. 10).
*Somerset.* Piggeries, Pedwell, Ashcott, Bridgwater (Aug. 4); Deal Farm, Catcott, Bridgwater; Hillside & Old Vicarage, Chilton Polden, Bridgwater (Aug. 5).
*Staffs.* Netherset Hey Farm, Madeley, Crewe (Aug. 6).
*Suffolk.* Silver Street, Withersfield, Haverhill (Aug. 5).
*Yorks.* Northfield Stud, High Burton, Huddersfield; R.A.F. Station, Dishforth, Thirsk (Aug. 10).

## DISEASES OF ANIMALS ACT, 1950—GREAT BRITAIN

**Summary of Returns of Confirmed Outbreaks of Scheduled (Notifiable) Diseases**

| Period | | Anthrax | Atrophic Rhinitis | Foot-and-Mouth | Fowl Pest | Sheep Scab | Swine Fever |
|---|---|---|---|---|---|---|---|
| 1st to 15th July, | 1959 ... ... | 5 | — | — | 7 | — | 37 |
| Corresponding period in | 1958 ... ... | 5 | — | — | 13 | — | 75 |
| | 1957 ... ... | 9 | — | 4 | 40 | — | 75 |
| | 1956 ... ... | 19 | 2 | — | 31 | — | 34 |
| 1st Jan. to 15th July, | 1959 ... ... | 132 | — | 9 | 424 | — | 638 |
| Corresponding period in | 1958 ... ... | 96 | 3 | 111 | 322 | — | 766 |
| | 1957 ... ... | 236 | 5 | 28 | 495 | — | 530 |
| | 1956 ... ... | 1,037 | 8 | 13 | 354 | — | 421 |

## TUBERCULOSIS (ATTESTED HERDS) SCHEMES

The number of Attested Herds, *i.e.*, herds officially certified as free from Tuberculosis as at 30th June, 1959, was as follows :—

| England | Wales | Scotland | Total (Great Britain) |
|---|---|---|---|
| 142,890 | 38,779 | 44,097 | 225,766 |

## TUBERCULOSIS ORDER OF 1938

The number of bovine animals slaughtered under the Tuberculosis Order of 1938 during the period 1st April to 30th June, 1959, was 37.

# Letters to the Editor

### Prevention of Enzootic Muscular Dystrophy by Selenium Administration

Sir,—The enzootic muscular dystrophy of suckling beef cattle in the north of Scotland (Sharman, 1954) occurs when the dams are fed rations low in tocopherol content, and can be prevented by administration of α-tocopherol either to the dams or to the calves (Blaxter & Sharman, 1953). The quantity of α-tocopherol necessary to prevent the disease is, however, much higher than that found effective when artificial rations are given. Three field experiments in successive years each with over 160 cattle showed that while 150 mg. α-tocopherol per calf were completely effective 10 mg. per calf were not: 20 mg. α-tocopherol per calf appeared to be the minimal prophylactic dose this being at least 5 times the minimal amount needed with artificial rations (Blaxter & McGill, 1955).

To account for the high tocopherol requirement of the calves investigations of dietary factors which might effect the tocopherol requirement were undertaken. It was shown that neither unsaturation of the dietary fats (Garton *et al.*, 1956), nor concommitant dietary deficiencies of iron (Blaxter *et al.*, 1957) or magnesium (Blaxter & Sharman, 1955) were involved. The prevention of liver necrosis in the rat (Schwarz & Foltz, 1957) and of exudative diathesis in the chick (Schwarz *et al.*, 1957), by selenium compounds and the finding that selenium as selenite is 500 times more active than is α-tocopherol in these respects suggested that selenium deficiency might be involved in the enzootic muscular dystrophy of cattle in north Scotland. In this regard, selenium as selenite has been found effective in preventing a comparable degenerative disease of the muscles of sheep (Muth *et al.*, 1958), but selenium as selenite or selenocystine was not effective in preventing muscle degeneration in vitamin E deficient rabbits (Hove *et al.*, 1958).

Twenty-five farms collaborated, each providing 1 to 4 replicates of the experiment. Each of the 58 replicates consisted of 3 calves, one a control which received no treatment, one which received 200 mg. α-tocopherol each week, and one which received 0.55 mg. anhydrous sodium selenite equivalent to 0.25 mg. selenium each day. This quantity corresponded to a dietary concentration of approximately 0.5 p.p.m. for the smallest and youngest animals. The results are given in the Table.

INCIDENCE OF MUSCULAR DYSTROPHY ON 25 FARMS

| | *No treatment* | *Tocopherol* | *Selenium* |
|---|---|---|---|
| Calves at risk | 58 | 58 | 58 |
| Number of cases of muscular dystrophy | 10 | 2 | 0 |

One of the control calves died after showing signs of severe muscular dystrophy. The diagnosis was confirmed at *post mortem*, the muscles being clearly dystrophic and containing less than one third the normal creatine content. Two mild cases of dystrophy occurred in animals given α-tocopherol. In both instances their dams had in previous years failed to rear their calves owing to the disease, this familial incidence not being uncommon. None of the calves given selenium developed the disease.

The results show that small amounts of selenium will prevent the muscular dystrophy of cattle in north Scotland, a disease which is also prevented by relatively large amounts of α-tocopherol. The farms concerned were on soils derived from arenaceous sands of old red sandstone origin which suggests that on comparable soils dietary deficiency of selenium might also occur. Such deficiency might well be masked by an adequate tocopherol intake as instanced by the absence of the disease on farms in the Moray Firth area which feed rations containing silage and green fodder and its limitation to those feeding turnips and straw, a ration which provides about one-tenth of the tocopherol of silage rations.

Yours faithfully,

G. A. M. SHARMAN.

North of Scotland Agricultural College,
Inverness.

K. L. BLAXTER.
R. S. WILSON.

Hannah Dairy Research Institute,
Kirkhill,
Ayr.

*June 18th*, 1959.

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### Intramuscular Carbon Tetrachloride in the Treatment of Acute Fascioliasis in Sheep

Sir,—In September, 1958, I was faced, like many other practitioners, with an outbreak of acute fascioliasis in a flock of 250 lambs. The pattern of the outbreak followed that of a typical acute infestation; 2 lambs died after a short period of illness lasting up to 12 hours, and on *post-mortem* examination showed the signs of severe fluke infestation.

Twelve more were very weak, and blood samples from these revealed the haemoglobin level to be below 5 grammes per cent. in all cases. These latter animals were in such an advanced stage of the disease that the administration of carbon tetrachloride by mouth would have been hazardous if not fatal to them. The intramuscular route was chosen in the belief that this would be less toxic to the animal. For this method of administration the carbon tetrachloride was suspended in medicinal liquid paraffin. After trial and error the optimum dose was observed to be 4 ml. of a 50 per cent. carbon tetrachloride and liquid paraffin mixture. This was injected deep into the hamstring muscles from a posterior direction.

For this treatment the substance must be administered in as clean and sterile conditions as possible. Immediately after the injection the animals show a transient lameness, tend to become recumbent, and remain so for about half an hour. It is important that they should not be disturbed during this time. We observed that during the half hour after injection there was a progressive rise in the pulse rate which reached a maximum in 15 to 17 minutes.

Response to treatment has been encouraging; in all flocks treated by this method deaths ceased 12 hours after treatment. Owners stated that the period of greatest improvement was from the fifth to the twelfth day. In extensive and severe outbreaks it seemed wise to repeat the injection after 21 days as the effect of the carbon tetrachloride wears off after this period.

During the period September, 1958, to February, 1959, 7,400 sheep were treated by this method with very satisfactory results. The mixture was cheap and easy to make, easily used, and the animals examined after slaughter showed no muscle reaction or residue at the site of the injection. As Mr. C. M. Edwards suggests, we hope this year to try the mixture as a preventive rather than a cure.

Yours faithfully,
JOHN A. PARRY.

Bishop House,
Brecon,
South Wales.

*August 2nd*, 1959.

**Parenteral Administration of Anthelmintics**

Sir,—The parenteral administration of anthelmintics is of special interest to countries such as Nigeria, where large numbers of animals are presented for treatment without adequate facilities for restraint. These treatments frequently have to be administered by lay assistants who are more experienced with inoculation than with drenching techniques.

Riek and Keith (1958) have shown that the organic phosphorus compound 0,-o-dimethyl-2,2,2-trichloro-I-hydroxymethyl phosphonate, now marketed by Farbenfabriken Bayer A.G. as "Neguvon," is a highly effective treatment for the removal of *Haemonchus placei* and *Oesophagostomum radiatum* from cattle when given by mouth at a dosage rate of 44 mg. per kg. bodyweight. When the dosage was increased to 110 mg. per kg. the drug was also highly effective against *Bunostomum phlebotomum* and *Cooperia* spp. Critical anthelmintic tests, as defined by Hall and Shillinger (1925), carried out at Vom showed that "Neguvon" had an anthelmintic efficiency of approximately 100 per cent. against these species and against *Trichuris globulosa* when given by mouth at the rate of 80 mg. per kg. bodyweight.

Three critical tests, using one animal each, were then carried out in which the drug was administered by subcutaneous injection as a 5 per cent. solution in water. The dosage rates were 60, 30, and 20 mg. per kg. bodyweight respectively. At the 60 and 30 mg. levels 97 to 100 per cent. of the *Bunostomum, Haemonchus, Oesophagostomum,* and *Trichuris* burdens were removed, but a high activity against *Cooperia* was obtained only at the 60 mg. dosage rate. At 20 mg. per kg. it was highly effective only against *Haemonchus, Oesophagostomum,* and *Trichuris.*

At the dosage rates investigated for subcutaneous injection no reactions were noted at the site of injection, but the test animal which was given the drug at 60 mg. per kg. showed symptoms of systemic intoxication one hour after treatment. The symptoms were severe and consisted of dyspnoea, inability to rise, muscular tremors, abdominal pain, profuse diarrhoea, marked depression of the pupil, and depression of the pulse rate. The symptoms persisted for at least 4 hours but by the following morning they had disappeared and the animal appeared normal. At each of the lower dosage rates the 2 test animals showed no clinical signs of intoxication. As a further test for untoward effect, 15 yearling cattle were then given the drug by subcutaneous injection at the 30 mg. per kg. dosage rate. Only one animal developed slight dyspnoea and passed soft faeces; the remainder showed no symptoms of intoxication.

"Neguvon" is the first drug which has been shown to be effective against *B. phlebotomum* in Nigerian cattle and, provided the problems of toxicity which are commonly associated with the use of organic phosphorus compounds can be overcome, it promises to have an important application in this country. Further work is in progress at Vom to determine the anthelmintic reliability and safety of "Neguvon" treatment by the subcutaneous route, and the results will be published in due course.

Yours faithfully,
R. P. LEE,
R. A. O. SHONEKAN.

Federal Department of Veterinary Research,
Vom,
Nigeria.

*July 31st*, 1959.

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### Peritoneal Tympany and Peptic Ulceration

Sir,—I have read with interest the article on Peritoneal Tympany and Peptic Ulceration by Cunningham *et al.* (1959), and the following two cases may help to clarify the picture still further. Both occurred in April, 1959.

*Case I*

*Subject.* An Ayrshire cow approximately 6 years old.

*History.* The animal had been perfectly normal up to 4 days before my first examination, when it had a severe black diarrhoea. This had cleared up quickly but the cow went off its food and the appetite was down to a few pounds of hay. The cow was due to calve in 10 days.

*Clinical findings.* The animal was in good condition. Rectal discharge of greenish mucus with a a few clots of blood was evident. The rectal temperature was 104° F., the pulse 70 per min. of good volume and rhythm. The abdomen was distended and very tense all over but there was no peritoneal or rumen tympany. The rumen felt solid and its movements were shallow. Rumen sounds were absent over the left costal area. The cow showed generalised pain on ballottement of the abdomen. Rectal examination revealed a full-term foetus apparently dead. (Treatment: 5 g. Dimycin.)

*Second day.* The animal was clinically similar to the previous day. (Treatment repeated.)

*Third day.* The cow looked much worse and was recumbent. It had a continuous grunt and tended to grind its teeth together. Some normal faeces were being passed though in small amounts. The temperature was 103° F. and the pulse 80 per minute but of poor volume. There was no tympany present. After a struggle the cow rose to its feet and was able to walk to a box, but with frequent rests.

*Fourth day.* The cow was generally similar in condition to the previous day but was unable to rise, the pulse had risen to 90 per minute and the volume and rhythm were worse than on the previous day. Vaginal examination revealed a partly opened cervix. The cow died later during the day—a week after the onset of the illness.

*Post mortem.* The abomasum was displaced to the left flank and was full of rumen-like contents which had disguised the displacement during life. Peptic ulceration had resulted in a gross peritonitis affecting the rumen, abomasum, diaphragm, and spleen with early adhesions.

*Case II*

*Subject.* Two-year-old Angus Cross bullock in fat condition.

*History.* The animal had appeared perfectly normal until the day before examination when its appetite disappeared completely.

*Clinical findings. First day.* The animal was bright and lively and apart from a tense, slightly painful abdomen showed little abnormality. Faeces were normal in amount and consistency. The rectal temperature was 102.5° F. Because of the very good condition of the animal no treatment was given, so that if there was no improvement the following day salvage might be attempted.

*Second day.* The animal was slightly duller, the eyes were injected, and palpation of the tense abdomen gave signs of generalised discomfort. There was no tympany. The temperature was the same as it had been on the previous day. Normal faeces in small amounts were still being passed, but the appetite was nil.

*Post mortem.* The carcase was examined at the abattoir and there was found a widespread, long-standing, anterior abdomen peritonitis, extending from, and enclosing, the spleen round and under the rumen/reticulum, and enclosing the abomasum and omasum. Food material was found spreading along between the fibrous peritoneal adhesions and the stomach walls. This food material was in most places well contained by the long-standing fibrous reaction which was an inch thick in places. The perforating ulcer from this case was ¼ inch in diameter and there were many healed ulcers on the abomasal lining, most of them pinhead in size.

The clinical findings in cases of ulceration of the abomasum seem to fall into three categories:—

(a) Acute perforation of an active ulcer with extensive peritonitis as in Case I, followed by death within 10 days.

(b) Chronic peritonitis in a wasting animal with signs of a vague digestive disturbance as described by Pinsent *et al.* (1955), Marr *et al.* (1955), and Cunningham *et al.* (1959).

(c) Chronic peritonitis of considerable duration becoming gradually more extensive. The peritonitis in these cases seems to be well controlled, as in Case II, and the animal shows little outward signs of ill health until the fibrous reaction begins to interfere with the digestive processes.

According to Cunningham *et al.* (1959), there is usually regression in human ulcer activity during pregnancy and exacerbation in lactation, but in the case described above the ulcer activity reached a maximum with perforation before lactation commenced, so that in the bovine animal the reverse may be the case. However, in the bovine animal, displacement of the abomasum seems to play some part in the pathogenesis, although in the case recorded by Pinsent *et al.* (1955), and in the bullock described above, there was no evidence of this displacement.

Yours faithfully,
COLIN J. ALLISON.

Ardmair,
St. Annes Road,
Dumfries.

*June 5th*, 1959.

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